

The University of Calgary, Department of Biology, Calgary, Canada

## Sensitivity of certain soil microbial processes to acid deposition

R. J. F. BEWLEY<sup>1)</sup> and D. PARKINSON

With 6 figures

(Accepted: 85-03-15)

### 1. Introduction

The decomposition and mineralization of specific organic residues in soil is effected by the activities of different groups of microorganisms. Inhibition of such processes by a particular environmental stress could have serious consequences for ecosystem functioning in terms of nutrient cycling and normal soil fertility. One such stress which has aroused particular concern is the acidification of soil by the dry and wet deposition of sulphur dioxide, SO<sub>2</sub>.

In recent years, there have been a number of studies concerning the effect of simulated acid rain (of which SO<sub>2</sub> is a precursor) on soil microbial activity (TAMM *et al.* 1977; BÅÅTH *et al.* 1979; HOVLAND *et al.* 1980; ROBERTS *et al.* 1980; HOVLAND 1981; STRAYER & ALEXANDER 1981; STRAYER *et al.* 1981; FRANCIS 1982; KILLHAM *et al.* 1983; BEWLEY & STOTZKY 1983a, b, c, d, 1984). However, it is difficult to draw firm conclusions as to the relative degree of inhibition of a particular acidic stress on microbial activity as workers have applied different intensities of acid input in different soil environments. Moreover, under field conditions, microbial populations have usually been subjected to a longer period of chronic, rather than an acute stress. BRYANT *et al.* (1979) detected differences in the relative degree of inhibition of the decomposition of a number of organic compounds in soil which had been acidified over several years by biological oxidation of sulphur from a stockpile, compared with that in soil acidified by addition of dilute sulphuric acid under laboratory conditions.

The impact of dry deposition of SO<sub>2</sub> on the soil microflora may differ from that of acid precipitation as the former could also include highly toxic anionic products such as bisulphite in addition to the proton input. Laboratory studies have demonstrated the toxic effects of gaseous SO<sub>2</sub> upon the mineralization of glucose and protein hydrolysate (GRANT *et al.* 1979), yet there is scant information concerning microbial activity in field sites impacted by dry deposition of this pollutant.

An important source of acidic pollution in Alberta, Canada, is the dry deposition of SO<sub>2</sub> released from "sour gas" plants, responsible for the removal of H<sub>2</sub>S from natural gas to produce a saleable product. Closer to one particular plant, there was increased input of SO<sub>2</sub>, and also sulphur dust which had resulted in acidification of the soil (LEGGE *et al.*, unpublished data). Under increasing acid deposition there was a reduction in microbial biomass, in basal respiration rates, and a retardation in the decomposition of glucose and vanillin (BEWLEY & PARKINSON 1986). There was also a decrease in total numbers of bacteria and starch-utilizing bacteria but an increase in the proportion of spore-formers (BEWLEY & PARKINSON 1984a). This was reflected in a decrease in the contribution of bacteria to total respiration relative to fungi, closer to the sour gas plant (BEWLEY & PARKINSON 1984b). No qualitative differences were detected in the mycoflora of decomposing pine needles closer to the emission source (BEWLEY & PARKINSON 1984b) or in the number of actively sporulating fungi, although a greater proportion of the latter were tolerant to either sulphite or bisulphite with

<sup>1)</sup> Corresponding author.

increasing SO<sub>2</sub> pollution (BEWLEY & PARKINSON 1984a). Field studies demonstrated a reduction in soil respiration and decomposition of pine needles together with an accumulation of litter, with increasing acid deposition (PRESCOTT & PARKINSON 1985).

The objectives of the present study were to extend these investigations to examine the effects of acid deposition upon the activities of microorganisms responsible for the mineralization of three substrates: cellulose, urea and casein. Cellulose was selected as it is the most abundant polymeric constituent of plant material (BURNS 1982), casein for examining the activities of proteolytic and ammonifying organisms (and comparing the relative degrees of inhibition of carbon and nitrogen mineralization) and urea, as an example of a compound used as a commercial fertiliser which is mineralized by a particular physiological group of organisms possessing a specific enzyme (urease).

## 2. Materials and methods

### 2.1. Site description

Three sites, previously defined as being "ecologically analogous" (LEGGE *et al.* 1981) were selected at 2.8 km (site 1), 6.0 km (site 2) and 9.6 km (site 3) downwind of a sour gas processing plant located 45 km west of Whitecourt, Alberta, Canada (54° 15' N, 116° W). The study area consisted of a transition of boreal and subalpine forest regions, the predominant tree species being lodgepole pine × jack pine hybrids (*Pinus contorta* LOUD. × *Pinus banksiana* LAMB.), and the soil type a degraded eutric brunisol. Respective concentrations of sulphate-sulphur at sites 1, 2 and 3 were 1860, 245 and 109 ppm in the organic layer, and 92, 14 and 3 ppm at 5 cm depth. A full description of sites 1, 2 and 3 (corresponding respectively to analogues A<sub>II</sub>, A<sub>III</sub> and A<sub>V</sub>) has been presented (LEGGE *et al.* 1981; BEWLEY & PARKINSON 1984a).

### 2.2. Decomposition of added substrates

Mineral soil samples to a depth of approximately 5 cm were removed from five random locations at each site. Organic debris (including roots) was carefully removed and the remaining sample passed through a 2 mm sieve. Five random samples of the organic layer were also removed from each site. Freshly fallen needles, plants, roots and lichens were removed from each sample so that only the F/H layer ("organic soil") remained. Determinations of pH (1:2 mineral soil: water/0.01 M CaCl<sub>2</sub>, 1:4 organic soil: water/0.01 M CaCl<sub>2</sub>), moisture content (loss in mass after drying at 75 °C for 20 h), total water capacity (immersing soil cores in water for 24 h, draining for one h, then determining the water retained), field capacity [333.3· hPa ( $\cong \frac{1}{3}$  bar) tension moisture content] and organic matter content (loss of mass on ignition at 400 °C for 24 h) were made on the five replicate samples of organic and mineral soil.

Prior to the determination of CO<sub>2</sub> evolution, sufficient water was added to raise the moisture content of each soil sample to predetermined optimal levels (field capacity for the organic soil, 50 of total moisture capacity for mineral soil). The samples of organic and mineral soil were then incubated at approximately 23 °C for 24 or 48 h respectively. Preliminary studies had indicated that there was no apparent inhibitory effect over the range of substrate levels tested, so concentrations of urea and cellulose which produced steady measurable increases in CO<sub>2</sub> production over several days were chosen. Cellulose (supplied by Baker Chemical Co., Phillipsburg, U.S.A. and ball-milled for 3 days), was added at concentrations of 0.2 g · 8 g organic soil<sup>-1</sup> and 0.5 g · 50 g mineral soil<sup>-1</sup>, and urea at concentrations of 0.08 g · 5 g organic soil<sup>-1</sup> and 0.4 g · 100 g mineral soil<sup>-1</sup>. Casein was added to the mineral soil at a concentration of 0.1 g · 10 g soil<sup>-1</sup> (i.e. 1 m/m [ $\cong$  w/w], predetermined as the optimal concentration). Cellulose was added to the organic soil as a water-based suspension, but in other cases the substrate was added as a finely ground powder with 0.2 g talc to the organic soil, 0.5 g talc to the mineral soil, to act as carrier. Hourly CO<sub>2</sub> output from each soil sample, incubated at 23 °C was determined using an "Ultragas" Gas Analyzer (Wösthoff Co., Bochum, F.R.G.). Basal respiration, prior to adding urea or cellulose was also determined.

To determine whether the ammonification of urea had significantly increased the soil pH and whether such conditions had resulted in a "recovery" of bacteria (reduced under acidic stress, BEWLEY & PARKINSON 1984a), the pH of the samples was determined at the end of the incubation period (1 days for the organic and 9 days for the mineral soil) and a dilution plate study was carried out. Soil suspensions were plated onto soil extract agar amended with 50 µg cyclohexamide ml<sup>-1</sup>, and spore forming bacteria were isolated on the same medium after heating the dilution tubes at 80 °C for 10 min. The procedure was as used in a previous study (BEWLEY & PARKINSON 1984a).

### 2.3. Microbial transformations of nitrogen

Five replicate samples of mineral soil per site (as used in the casein decomposition study) were remoistened to 50 of their total moisture capacity and stored at 23 °C for three days. Casein was

added at a concentration of 1% (m/m) along with an equal quantity of talc. Approximately 40 to 50 g of soil from each sample was placed in each of five 125 ml Erlenmeyer flasks and a further subsample was used for immediate sampling (day 0). The flasks were stoppered with foam rubber plugs, weighed, and incubated in darkness at 23 °C. Sufficient sterile water was added as necessary during the incubation period to restore soil moisture levels to their original concentrations. One set of flasks was removed for sampling after 2, 4, 7, 15 and 24 days. A set of samples without added casein was also incubated under identical conditions and sampled after 0 and 24 days. On each sampling occasion, 11 to 12 g of soil was accurately weighed into 100 ml of 2M KCl and shaken vigorously for one hour (KEENEY & NELSON 1982). The samples were centrifuged at 12,500 RPM for 20 min, decanted, and a few drops of chloroform added. Each sample of extractant was filtered under suction through a No. 42 "Whatman" filter paper (Whatman Ltd., England), and stored at 3 °C prior to analysis. Analysis for ammonium nitrogen ( $\text{NH}_4\text{-N}$ ) and total oxidized nitrogen ( $\text{NO}_2\text{-N}$  and  $\text{NO}_3\text{-N}$ ) was carried out using a Technicon Auto Analyzer II.

## 2.4. Field study of cellulose decomposition

To determine the relative decomposition rate of cellulose *in situ*, the method of TATSUYAMA *et al.* (1981) was employed. Strips of polyethylene-backed absorbent paper ("Benchkote", made by Whatman Ltd., England) were attached to plastic-coated paper sheets (Nalgene "Poly Paper", Nalge Co., Rochester, N. Y., U.S.A.) for support, the paper side of each strip facing outwards. In May 1983, 25 sets of three strips were randomly buried lengthwise at each site, so that the top of a 10 cm  $\times$  4 cm area marked on each strip was approximately 2 cm below the surface of the litter. The strips were carefully removed one year 16 days later, transported to the laboratory and dried at 30 °C for eight days. From two of the three strips comprising each set, visible organic debris was removed with forceps, the 10 cm  $\times$  4 cm area marked on each strip was cut off, weighed, and heated in a muffle furnace at 400 °C for 24 h. To test the effects of washing, the third strip was rinsed in tap water using a fine spray, dried at 80 °C for 15 h, and weighed before placing in the muffle furnace. Further weighed control strips of 10 cm  $\times$  4 cm were soaked in concentrated  $\text{H}_2\text{SO}_4$ , washed in tap water and dried at 80 °C to estimate the mass of the polyethylene backing, and hence the original mass of the cellulose. The mass loss of each buried strip on ignition minus the mass of the polyethylene backing was used to calculate the mass of cellulose remaining after burial, and hence the percentage of cellulose decomposed.

## 2.5. Statistical analysis of data

To test for inter-site differences, data sets were subjected to a one-way analysis of variance, and if significant, confidence limits for differences between means were determined. Prior to the analysis of variance, a Bartlett's test of homogeneity of variance was performed, and where necessary, logarithmic or arcsine transformations of the data were carried out. If the data was not transferable, a Kruskal-Wallis analysis was used to test for inter-site differences and where significant, a non-parametric multiple comparison (using rank sums) was performed to determine between which of the three sites significant differences occurred (ZAR 1974).

# 3. Results

## 3.1. Soil pH, organic matter content, and basal respiration rates

There was a decrease in soil pH with decreasing distance from the gas plant: in both the organic and mineral soil horizons the pH of site 1 was approximately 1 to 1.5 units lower than that of sites 2 and 3 (Table 1). All three sites had similar organic matter contents in both the organic and mineral soil horizons, but there was a lower basal respiration rate at site 1, compared with sites 2 and 3. These data are consistent with the results of previous studies (BEWLEY & PARKINSON 1984a, 1986). The data presented in Table 1 refer to the soils which were used to study cellulose decomposition: similar data were also obtained for the soils used to study urea decomposition, except on this occasion no significant reduction in basal respiration in the mineral soil was detected with increasing acidic stress.

## 3.2. Field Study of cellulose decomposition

There was a small, but highly significant ( $P < 0.001$ ) reduction in cellulose decomposition in the field, with increasing acidic deposition. The estimated percentages of cellulose decomposed at sites 1, 2 and 3 on the unwashed plastic-backed strips were  $83 \pm 1.8$ ,  $91 \pm 0.8$ , and  $93 \pm 0.4$ , respectively (mean of 50 samples  $\pm$  S.E.M.). The difference between any one

Table 1. pH, organic matter content and basal respiration rates of soil collected 2.8 km (site 1), 6.0 km (site 2) and 9.6 km (site 3), from a sour gas plant emitting SO<sub>2</sub> (mean of 5 samples  $\pm$  S.E.M.)

	Site 1			Site 2			Site 3		
<b>Property of organic soil</b>									
% organic matter	91	$\pm 2.1$	a <sup>1)</sup>	87	$\pm 3.0$	a	83	$\pm 3.4$	a
pH (1: 4 soil : H <sub>2</sub> O)	2.8	$\pm 0.06$	a	3.8	$\pm 0.06$	b	4.5	$\pm 0.11$	c
pH (1: 4 soil: CaCl <sub>2</sub> )	2.6	$\pm 0.04$	a	3.6	$\pm 0.06$	b	4.0	$\pm 0.09$	c
Basal respiration rate (mg C · 100 g <sup>-1</sup> h <sup>-1</sup> )	1.9	$\pm 0.19$	a	6.1	$\pm 0.55$	b	7.4	$\pm 0.60$	b
<b>Property of mineral soil</b>									
% organic matter	1.7	$\pm 0.24$	a	1.9	$\pm 0.09$	a	2.0	$\pm 0.05$	a
pH (1: 2 soil: H <sub>2</sub> O)	4.3	$\pm 0.06$	a	5.2	$\pm 0.11$	b	5.9	$\pm 0.19$	c
pH (1: 2 soil: CaCl <sub>2</sub> )	3.5	$\pm 0.12$	a	4.2	$\pm 0.12$	b	4.9	$\pm 0.20$	c
Basal respiration rate (mg C · 100 g <sup>-1</sup> h <sup>-1</sup> )	0.042	$\pm 0.0039$	a	0.073	$\pm 0.0097$	b	0.097	$\pm 0.0052$	b

<sup>1)</sup> Means followed by the same letter do not differ significantly at  $P < 0.05$ .

of the three sites and any other was significant at  $P < 0.01$  (as tested by a non-parametric multiple comparison). On the washed strips, the estimated percentage of cellulose decomposed was slightly higher at each site:  $86 \pm 2.6$  at site 1,  $93 \pm 1.1$  at site 2 and  $95 \pm 0.4$  at site 3 (mean of 25 samples  $\pm$  S.E.M.), and on this occasion, all differences between sites were significant at  $P < 0.05$ .

### 3.3. Mineralization of cellulose

There was a lower rate of CO<sub>2</sub> evolution from cellulose-amended organic soil from site 1, compared with that from sites 2 and 3 (Fig. 1). The total mg of carbon evolved as CO<sub>2</sub> after a 210 h incubation period were  $38 \pm 3.1$  from site 1,  $115 \pm 8.9$  from site 2, and  $130 \pm 10.8$  from site 3 (mean  $\pm$  S.E.M.); the difference between site 1 and sites 2 or 3 was significant ( $P < 0.001$ ) but not that between sites 2 and 3 ( $P > 0.05$ ). In cellulose-amended mineral soil, however, there was a significant difference in carbon mineralization between site 3 and both sites 1 and 2 ( $P < 0.05$ ) after 20 d of incubation, whereas the difference between sites 1 and 2 was not significant (Fig. 2). The total mg carbon mineralized at the end of this period was  $14 \pm 1.1$ ,  $18 \pm 3.1$  and  $27 \pm 1.9$  for sites 1, 2 and 3, respectively.

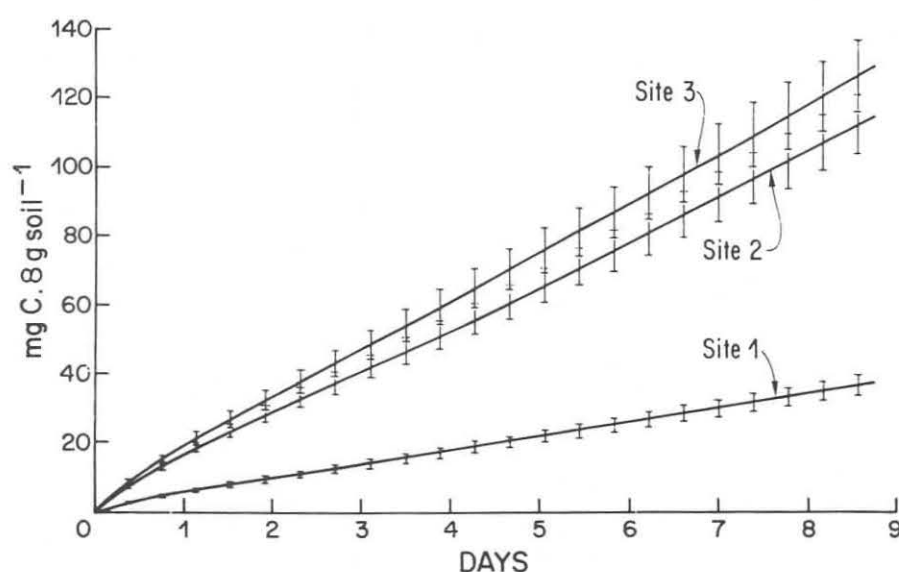


Fig. 1. Total carbon evolved as CO<sub>2</sub> from cellulose-amended (2.5%) organic soil collected 2.8 km (site 1), 6.0 km (site 2) and 9.6 km (site 3) from a sour gas plant emitting SO<sub>2</sub> ( $\bar{x} \pm$  S.E.M.).

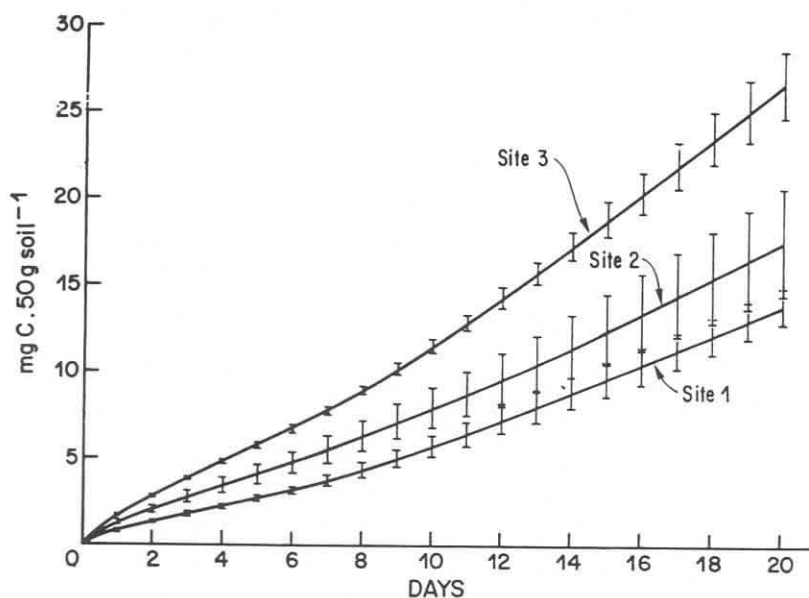


Fig. 2. Total carbon evolved as  $\text{CO}_2$  from cellulose-amended (1%) mineral soil collected 2.8 km (site 1), 6.0 km (site 2) and 9.6 km (site 3) from a sour gas plant emitting  $\text{SO}_2$  ( $\bar{x} \pm \text{S.E.M.}$ ).

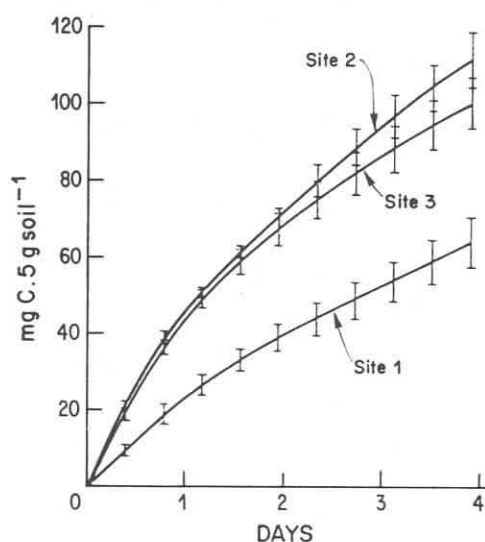


Fig. 3. Total carbon evolved as  $\text{CO}_2$  from urea-amended (1.6%) organic soil collected 2.8 km (site 1), 6.0 km (site 2) and 9.6 km (site 3) from a sour gas plant emitting  $\text{SO}_2$  ( $\bar{x} \pm \text{S.E.M.}$ ).

### 3.4. Mineralization of urea

Throughout the incubation period there was significantly less ( $P < 0.001$ ) urea mineralization in site 1 organic soil compared with sites 2 and 3, but no significant differences between these latter sites (Fig. 3). The total amount of carbon evolved as  $\text{CO}_2$  from urea-amended mineral soil of site 1 was significantly less ( $P < 0.05$ ) than that from both sites 2 and 3 after 1, 2 and 3 days of incubation (Fig. 4). On days 4 and 5, however, site 1 differed significantly only from site 3 ( $P < 0.05$ ) and from day 6 onwards there were no differences in the total carbon mineralized between the three sites.

The initial  $p\text{H}$  levels of both the organic and mineral soils were similar to the values presented in Table 1 for each of the three sites, and addition of urea initially only resulted in a slight increase in the  $p\text{H}$  of both organic and mineral soils of 0 to 0.6 units. After 4 days, however, the  $p\text{H}$  (1:5, soil: water) of the organic soil was  $6.9 \pm 0.24$  for site 1,  $7.0 \pm 0.07$  for site 2, and  $7.3 \pm 0.16$  for site 3 (mean  $\pm \text{S.E.M.}$ ). After 9 days, the  $p\text{H}$  of the mineral soil (1:2, soil: water) was  $7.9 \pm 0.13$  for site 1,  $8.5 \pm 0.22$  for site 2, and  $8.2 \pm 0.11$  for site 3. These differences between sites were not significant ( $P > 0.05$ ).

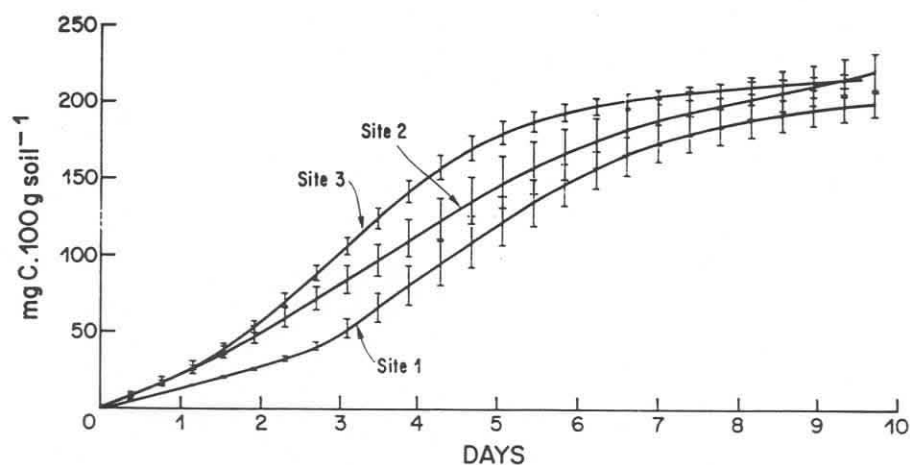


Fig. 4. Total carbon evolved as  $\text{CO}_2$  from urea-amended (0.4%) mineral soil collected 2.8 km (site 1), 6.0 km (site 2) and 9.6 km (site 3) from a sour gas plant emitting  $\text{SO}_2$  ( $\bar{x} \pm \text{S.E.M.}$ ).

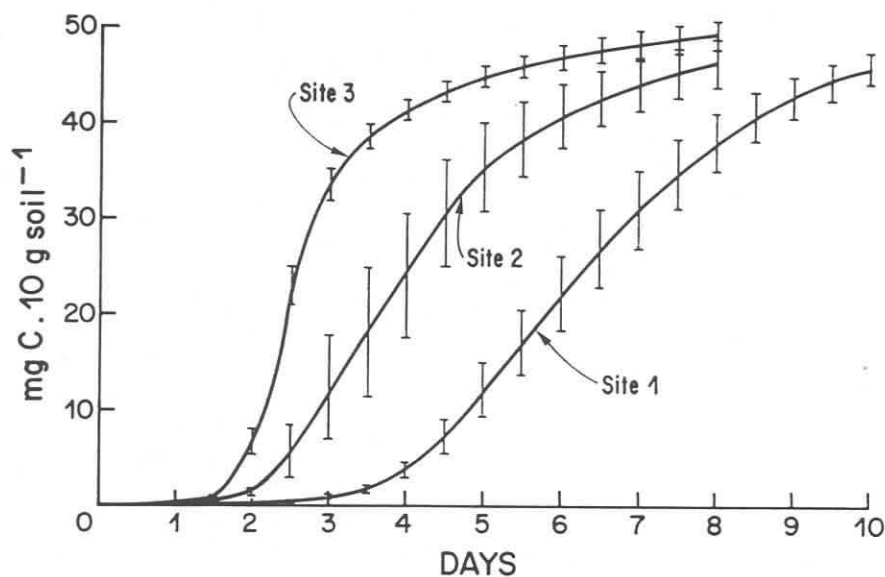


Fig. 5. Total carbon evolved as  $\text{CO}_2$  from casein-amended (1%) mineral soil collected 2.8 km (site 1), 6.0 km (site 2) and 9.6 km (site 3) from a sour gas plant emitting  $\text{SO}_2$  ( $\bar{x} \pm \text{S.E.M.}$ ).

Following incubation with urea, significantly fewer bacteria  $\cdot \text{g organic soil}^{-1}$  ( $P < 0.01$ ) were isolated from site 1 ( $90 \pm 46.7 \times 10^7$ ) compared with sites 2 ( $448 \pm 49.4 \times 10^7$ ) and 3 ( $492 \pm 95.8 \times 10^7$ ), (mean  $\pm \text{S.E.M.}$ ). The mean numbers of bacteria isolated from urea-, amended mineral soil  $\times 10^7 \text{ g}^{-1}$  were  $11 \pm 4.0$ ,  $17 \pm 3.4$  and  $13 \pm 4.7$  for sites 1, 2 and 3 respectively: differences between sites were not significant ( $P > 0.05$ ). No significant differences were detected in numbers of spore-forming bacteria isolated from both the organic and mineral soil samples.

### 3.5. Mineralization of casein

There was a considerable retardation in the decomposition of casein with increasing presubjection to acid deposition (Fig. 5). Significantly less ( $P < 0.05$ )  $\text{CO}_2$  was evolved from site 1 soil compared with site 3 throughout the incubation period, and also compared with site 2 from days 1 through 7. On days 2, 3 and 4, the total  $\text{CO}_2$  evolved from site 2 soil was also significantly below that of site 3 ( $P < 0.05$ ), whereas on other occasions, the differences between these two less contaminated sites were not significant ( $P > 0.05$ ). These differences in carbon mineralization, at least in the early stages, were a reflection of both a longer lag before significant casein mineralization occurred and also, once initiated, a lower rate of decomposition with increasing acidic stress. The time (in hours) taken for peak  $\text{CO}_2$  efflux

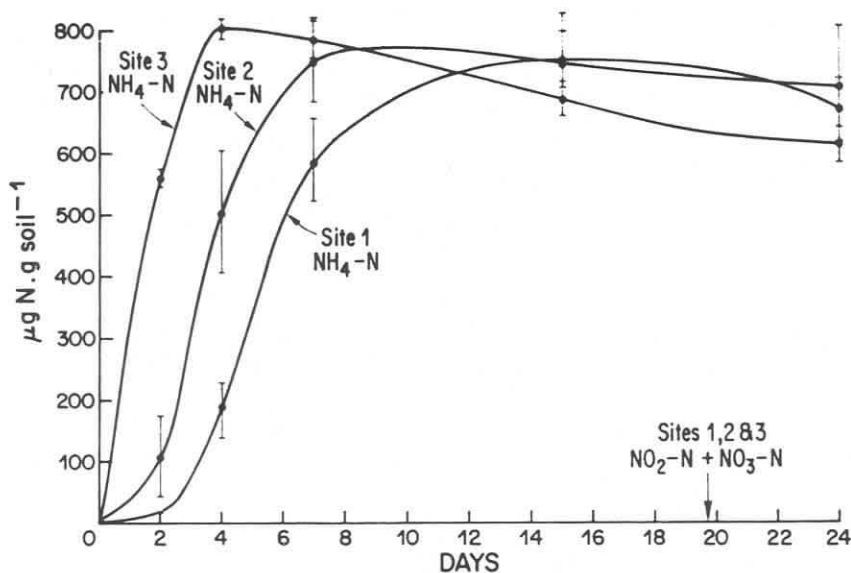


Fig. 6. Extractable ammonium-nitrogen ( $\text{NH}_4\text{-N}$ ) and oxidized nitrogen ( $\text{NO}_2\text{-N} + \text{NO}_3\text{-N}$ ) from casein-amended (1%) mineral soil collected 2.8 km (site 1), 6.0 km (site 2) and 9.6 km (site 3) from a sour gas plant emitting  $\text{SO}_2$  ( $\bar{x} \pm \text{S.E.M.}$ ).

to be attained:  $137 \pm 11.6$  for site 1,  $91 \pm 11.1$  for site 2 and  $55 \pm 1.6$  for site 3 (mean  $\pm \text{S.E.M.}$ ), is a function of both these factors, all differences between sites being significant ( $P < 0.05$ ). There was no significant difference between the peak hourly  $\text{CO}_2$  output per 10 g sample of soil for site 1 ( $0.57 \pm 0.046 \text{ mg C}$ ) and site 2 ( $1.04 \pm 0.162 \text{ mg C}$ ) although both were significantly less ( $P < 0.05$ ) than from site 3 ( $1.59 \pm 0.153 \text{ mg C}$ , mean  $\pm \text{S.E.M.}$ ).

### 3.6. Nitrogen transformations

On day 0, the concentrations of  $\text{NH}_4\text{-N}$  as ppm ( $\mu\text{g g}^{-1}$ ) in unamended soil were  $0.72 \pm 0.301$ ,  $0.71 \pm 0.135$ , and  $0.90 \pm 0.066$  for sites 1, 2 and 3, respectively (mean  $\pm \text{S.E.M.}$ ). After 24 days these concentrations increased to  $4.0 \pm 1.22$ ,  $5.2 \pm 2.63$ , and  $8.7 \pm 2.72$  ppm, respectively. None of these differences between sites were significant ( $P > 0.05$ ). Nitrate plus nitrite was not detectable in soil from sites 1 or 2 on day 0, and even in site 3 soil, the concentration of  $(\text{NO}_3 + \text{NO}_2)\text{-N}$  was only  $0.05 \pm 0.043$ . After 24 days of incubation, low levels of  $(\text{NO}_3 + \text{NO}_2)\text{-N}$  were detected in some samples from site 2 (mean concentration,  $0.15 \pm 0.149 \text{ ppm}$ ) and site 3 (mean concentration,  $0.96 \pm 0.96 \text{ ppm}$ ), but not from site 1.

Active ammonification of added casein took place in all three soils, although there was a decrease in the  $\text{NH}_4\text{-N}$  formed in the more polluted soils in the early stages (Fig. 6). Significantly less  $\text{NH}_4\text{-N}$  was detected in site 1 soil compared with sites 2 and 3 on days 2 and 4, but the reduction in ammonium formation in site 2 soil compared with site 3 soil was significant only on day 4 ( $P < 0.05$ ). On all other occasions, no significant differences in  $\text{NH}_4\text{-N}$  were detected between sites. There was a slight decrease in  $\text{NH}_4\text{-N}$  in soils from all three sites towards the end of the incubation period. However, there was no evidence of nitrification: throughout the incubation period mean concentrations of  $(\text{NO}_3 + \text{NO}_2)\text{-N}$  did not exceed 1 ppm, at each site.

## 4. Discussion

The  $\text{CO}_2$  released from the soil following amendment with any of the substrates tested resulted from both the decomposition of that substrate and the organic matter already present in the soil. In this study, no attempt was made to partition these two sources, as the addition of the substrate could itself enhance the decomposition of the existing organic material, by direct stimulation of the microflora or by alteration of pH. If, for example, the basal respiration rate prior to adding urea was subtracted from each hourly value for

CO<sub>2</sub> output following amendment, then the cumulative amount of carbon evolved as CO<sub>2</sub> after 9 days from site 3 was 193 mg whereas only 80 mg C were added as urea.

In at least the early stages of the decomposition of all the substrates tested there was a reduction in carbon and nitrogen mineralization in soil from site 1, compared with site 3, and sometimes, compared with site 2. This was consistent with previous findings indicating a retardation in the decomposition of glucose and vanillin, together with reduced bacterial numbers and microbial biomass at this site (BEWLEY & PARKINSON 1984a, b, c). There was often a reduction in mineralization at site 2 compared with site 3, and the extent of this provides some indication of the relative sensitivity of the process involved. In the mineral, but not in the organic soil, the rate of CO<sub>2</sub> evolution from cellulose-amended soil from site 2 was significantly less, and only 66% of that from site 3 after 20 d, in contrast to the processes of urea and casein decomposition, both of which "recovered" towards the end of the incubation period. The reduction in CO<sub>2</sub> evolution from cellulose-amended organic soil of site 2 (approximately 89% of site 3), was not significant, possibly due to the "masking effect" of CO<sub>2</sub> released from the large amount of organic matter present, relative to the small amount of added substrate. Nevertheless, the total amount of carbon mineralized from site 1 organic soil was only 29% of that from site 3 after 9 days of incubation.

Cellulose decomposition is known to be a pH-sensitive process (WHITE *et al.* 1949; SCHMIDT & RUSCHMEYER 1958; RUSCHMEYER & SCHMIDT 1958), and there have been several investigations concerning its response to acid precipitation (both simulated and field). There was a small decrease in the extractable amount of cellulase in samples of L and F horizons of low pH following treatment in the field with simulated acid rain, and approximately 25% of the variation in cellulase activity was explained by litter pH (HOVLAND 1981). Prior fumigation of a forest soil of pH 4 with 1.0  $\mu$ l SO<sub>2</sub> l<sup>-1</sup> for 48 h reduced cellulose decomposition, but not at a statistically significant level (GRANT *et al.* 1979a). Field studies of cellulose decomposition under acid deposition comparable with the present investigation however are few. LANGKRAMER & LETTL (1981) reported a delay in cellulose decomposition in soils impacted by industrial emissions containing high concentrations of SO<sub>2</sub>. Transfer of an unpolluted soil of pH 4.2 to an area impacted by sulphur emissions (125  $\mu$ g SO<sub>2</sub> m<sup>-3</sup>) reduced the pH to 3.7 but had no significant effect on cellulase activity (WAINWRIGHT 1980). BRYANT *et al.* (1979) observed a higher rate of CO<sub>2</sub> evolution from soil of pH 6.8 taken 200 m from a sulphur stockpile compared with both the same soil acidified to pH 2.9 *in vitro* and with soil of pH 3.0 collected 1 m from the stockpile, following enrichment with cellulose. Although the rate of CO<sub>2</sub> evolution from urea-enriched soil was also reduced under both laboratory and field conditions, it still exceeded CO<sub>2</sub> evolution rates from corresponding soil samples without urea. In contrast, CO<sub>2</sub> evolution rates from cellulose-amended, acidified soils were similar to those from corresponding soils without cellulose. The authors concluded that the microflora responsible for degrading cellulose was more acid-sensitive than the urea-metabolizing organisms, a finding therefore consistent with the results of the present study.

Although the difference in decomposition of the paper strips buried at sites 2 and 3 was small in magnitude, it was nonetheless significant. The results from the unwashed strips probably give a more accurate estimate of the actual loss of cellulose, as washing tended to remove loose fragments of partially decomposed cellulose in addition to any remaining organic matter. The 10% reduction in cellulose loss at site 1 compared with site 3 was smaller in magnitude than the corresponding inter-site differences in CO<sub>2</sub> evolution from cellulose-amended soils. It is possible that the decomposition of naturally occurring cellulose may be inhibited to a greater extent by acid deposition than that of these buried paper strips. The plant material has itself been subjected to the effects of sulphur emissions over several years of exposure, so the toxicant may be intimately associated with the substrate. (The extremely fine nature of the ball-milled cellulose allowed for such intimate association with the soil matrix in the respirometric studies.) Also, most of the area of the strips was exposed to the mineral soil where the degree of acid deposition was smaller than in the L, F and H horizons. Finally, the association of cellulose with other, more recalcitrant lignin materials in plant litter may alter the susceptibility of the overall decomposition process to acid deposition.

Notwithstanding these problems of direct extrapolation, the buried strip method appears to be quite sensitive for detecting changes in cellulose decomposition effected by acid deposition. Over a 7 month period, there was also a small, but significant, reduction in weight loss of cellulose powder mixed into soil of pH 3.3 downwind from a coking plant, polluted by acid rain, sulphur, phenolic and naptha compounds, compared with that left in soil of pH 4.9 at an unpolluted site (KILLHAM & WAINWRIGHT 1981). Conversely, reductions in tensile strength of cottontuck strips buried in soil having different pH values and concentrations of cadmium, lead and zinc, were not significantly different (MOLONEY *et al.* 1983).

Although the organic horizon was more impacted by the effects of acid deposition than the mineral soil below (as indicated by higher sulphate-sulphur concentrations and lower pH levels), it contained a higher number of microorganisms (BEWLEY & PARKINSON 1984a), so a greater probability of there being species capable of degrading specific substrates might be expected. There was no difference in the degradation rate of urea between sites 2 and 3 in the organic soil, yet after 4 days, the amount of carbon mineralized, and numbers of bacteria isolated from site 1, was still significantly lower, despite the pH of the soil having increased to 7, similar to that of the other sites. These findings suggest that the previous impact of acid deposition was of a severity such that the urea mineralizing microflora of the organic layer did not recover as soon as conditions were ameliorated. Previous studies had indicated that reductions in the basal respiration rate and in bacteria from the mineral soil of site 1, were somewhat inconsistent (BEWLEY & PARKINSON 1984a, b; PRESCOTT & PARKINSON 1985). However, it is notable that there was a significant retardation in urea decomposition despite the fact that on this occasion, the basal respiration was not significantly less than that at the other two sites.

There were greater differences in the mineralization of casein compared with urea, between sites (Fig. 5 *cf.*, Fig. 4). Urea is a low molecular mass substrate and the functional location of urease, the enzyme responsible for its degradation, is cytoplasmic (although it can continue to function extracellularly in cell debris or colloidal complexes; BURNS 1982). Conversely, casein and cellulose are both large molecular mass compounds which are decomposed by extracellular proteolytic and cellulolytic enzymes respectively. It is therefore possible that not only are the organisms responsible for the synthesis of these enzymes susceptible to acid deposition, but the acidic environment into which the enzymes are released, may itself inhibit enzyme activities. Thus at site 1, the longer lag phase before CO<sub>2</sub> evolution was initiated and the lower rate of casein decomposition, once underway, may reflect the degree of inhibition of proteolytic enzyme synthesis and activity, respectively. The addition of lead to a sandy soil substantially increased the lag phase prior to oxidation of both cellulose and starch, although this was less pronounced with glucose (DOELMAN & HAANSTRA 1979). As in the present study, the authors concluded that the pollutant was interacting with the cellulase and amylase released, or directly inhibiting enzyme synthesis or other activities of the microorganisms concerned.

After 2 d of incubation, there was little difference in the relative degree of inhibition of carbon and nitrogen mineralization from added casein. On day 4, the respective amounts of carbon mineralized as CO<sub>2</sub> in site 2 and site 1 soil, as percentages of that in site 3 soil were 58 and 9 compared with respective values of 63 and 23 for nitrogen mineralized as NH<sub>4</sub>. After 7 d of incubation, the respective amounts of carbon mineralized at sites 2 and 1 were 91% and 64% of site 3, compared with 96% and 75% for nitrogen mineralization. The apparently greater sensitivity of carbon versus nitrogen mineralization may be because the former process directly measures the activity of all organisms (proteolytic and ammonifying), whereas the ammonium concentration represents the activity of the ammonifiers. Obviously, any inhibition of proteolysis will also limit ammonification, so that though there are differences in sensitivity between carbon and nitrogen mineralization, they are small in magnitude. Ammonification of glycine was also retarded by simulated acid rain in a perfusion study, where the initial pH of the perfusate was sequentially lowered from 6 to 3, 2.5 or 2.0. As in the present study, however, recovery eventually took place, so that even with an initial pH of 2.8, 80% of the glycine was eventually ammonified over a 50 day period of incubation.

(BEWLEY & STOTZKY 1983d). Ammonium formed in incubated samples of a forest sandy loam treated with  $\text{H}_2\text{SO}_4$  to lower their pH to 3.2 was about 50% of that in control samples of pH 4.6, over a 180 d incubation (FRANCIS 1982). Little nitrification occurred in samples of either soil, except when the pH of the control was raised to 7.1 by addition of  $\text{Ca}(\text{OH})_2$ . In the present study it is perhaps surprising that no nitrification was detected in site 3 soil as the initial pH was around 6, and increased to over 8 following ammonification of casein (as with sites 1 and 2). This is in contrast to the forest soil studied by FRANCIS, where nitrifiers were present, but not active, until the pH was raised. In view of the absence of nitrification it is not clear what was responsible for the decrease in  $\text{NH}_4\text{-N}$  observed in all soils towards the end of the incubation period. It is possible that some  $\text{NH}_4\text{-N}$  was being assimilated into microbial biomass and therefore converted into an organic form, alternatively, some may have been lost through volatilization.

## 5. Conclusions

The process of cellulose decomposition was considerably slower than that of the other, more labile substrates examined in this investigation and inter-site differences were still detectable at the end of the incubation period. In the mineral soil at least, the processes of carbon and nitrogen mineralization of casein or urea appeared to "recover" after the initial inhibition, reflected by a longer lag and/or slower decomposition rates. The major objection to directly extrapolating a quantitative reduction in a particular microbial process observed *in vitro* to the field, is that these studies were performed under optimal conditions of substrate concentration, moisture, and temperature. Sub-optimal physical conditions in the field, together with temporal discontinuities in substrate availability are likely to enhance the inhibitory effects of acid deposition on enzyme synthesis. Such retardation in enzyme production by the particular group of organisms involved (which may also be reduced in number by innate sensitivity to acid deposition) are likely to result in far greater "lag" periods in the field than under laboratory conditions. A greater degree of inhibition of enzyme activity by acidity, and hence the rate of decomposition in the field may also be expected, as a result of prevailing sub-optimal physical conditions. The net effect of these factors will be a reduction in litter turnover and in the availability of nutrients to primary producers. Deficiencies of trace elements might also be expected, as their release into the soil environment is intimately associated with the mineralization of organic materials and a general reduction in microbial activity is likely to be reflected in an overall decrease in soil fertility.

## 6. Acknowledgements

This study was supported by a grant from the Natural Sciences and Engineering Research Council of Canada. We are grateful to Dr. A. H. LEGGE for assistance and information, PAT MAZIER and LEA BURTON for technical assistance, BARRY TAYLOR for statistical advice, and to the AMOCO Canada Petroleum Company Ltd., for access to their land.

## 7. References

- BÄÄTH, E., B. LUNDGREN, & B. SÖDERSTRÖM, 1979. Effects of artificial acid rain on microbial activity and biomass. *Bull. Environ. Contam. Toxicol.* **23**, 737—740.
- BEWLEY, R. J. F., & G. STOTZKY, 1983a. Simulated acid rain ( $\text{H}_2\text{SO}_4$ ) and microbial activity in soil. *Soil Biol. Biochem.* **15**, 425—429.
- 1983b. Anionic constituents of acid rain and microbial activity in soil. *Soil Biol. Biochem.* **15**, 431—437.
- 1983c. Effects of combinations of simulated acid rain and cadmium or zinc on microbial activity in soil. *Environ. Res.* **31**, 332—339.
- 1983d. Effects of cadmium and simulated acid rain on ammonification and nitrification in soil. *Arch. Environ. Contam. Toxicol.* **12**, 285—291.
- 1984. Degradation of vanillin in soil-clay mixtures treated with simulated acid rain. *Soil Sci.* **137**, 415—418.

- & D. PARKINSON, 1984a. Effects of sulphur dioxide pollution on forest soil microorganisms. *Can. J. Microbiol.* **30**, 179—185.
- — 1984b. Bacterial and fungal activity in sulphur dioxide-polluted soils. *Can. J. Microbiol.* **31**, 13—15.
- — 1984c. Monitoring the impact of acid deposition on the soil microbiota using glucose and vanillin decomposition. *Water Air Soil Pollut.* (submitted for publication).
- BRYANT, R. D., E. A. GORDY, & E. J. LAISHLEY, 1979. Effect of soil acidification on the soil microflora. *Water Air Soil Pollut.* **11**, 437—444.
- BURNS, R. G., 1982. Carbon mineralization. In: BULL, A. T., & J. H. SLATER (eds.), *Microbial Interactions and Communities*, Volume I. Academic Press, New York, 475—543.
- DOELMAN, P., & HAANSTRA, 1979. Effects of lead on the decomposition of organic matter. *Soil Biol. Biochem.* **11**, 481—485.
- FRANCIS, A. J., 1982. Effects of acidic precipitation and acidity on soil microbial processes. *Water Air Soil Pollut.* **18**, 375—394.
- GRANT, I. F., K. BANCROFT & M. ALEXANDER, 1979. Effect of SO<sub>2</sub> and bisulfite on heterotrophic activity in an acid soil. *Appl. Environ. Microbiol.* **38**, 78—83.
- HOVLAND, J., 1981. The effect of artificial acid rain on respiration and cellulase activity in Norway Spruce Needle Litter. *Soil. Biol. Biochem.* **13**, 23—26.
- G. ABRAHAMSEN & G. OGNER, 1980. Effects of artificial acid rain on decomposition of spruce needles and on mobilisation and leaching of elements. *Plant Soil* **56**, 365—378.
- KEENEY, D. R., & D. W. NELSON, 1982. Nitrogen-inorganic forms. In: PAGE, A. L., R. H. MILLER & D. R. KEENEY (eds.), *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*. 2nd Edit. American Society of Agronomy, Madison, 643—698.
- KILLHAM, K., & M. WAINWRIGHT, 1981. Deciduous leaf-litter and cellulose decomposition in soil exposed to heavy atmospheric pollution. *Environ. Pollut. Ser. A*. **26**, 79—85.
- M. K. FIRESTONE & J. G. MCCOLL, 1983. Acid rain and soil microbial activity: effects and their mechanisms. *J. Environ. Qual.* **12**, 133—137.
- LANGKRAMER, O., & A. LETTL, 1981. Changes of ecological conditions in soil of Norway Spruce forest stand harmed by industrial air pollution. *Prace. Vulhm.* **59**, 31—48.
- LEGGE, A. H., D. R. JAKUES, G. W. HARVEY, H. R. KROUSE, H. M. BROWN, E. C. RHODES, M. NOSAL, H. U. SCHELLHASE, J. MAYO, A. P. HARTGERINK, P. F. LESTER, R. G. AMUNDSON & R. B. WALKER, 1981. Sulphur gas emissions in the boreal forest; the West Whitecourt case study I. Executive Summary. *Water Air Soil Pollut.* **15**, 77—85.
- MOLONEY, K. A., L. J. STRATTON & R. M. KLEIN, 1983. Effects of simulated acidic, metal-containing precipitation on coniferous litter decomposition. *Can. J. Bot.* **61**, 3337—3342.
- PRESCOTT, C. E., & D. PARKINSON, 1985. Effects of sulphur pollution on rates of litter decomposition in a pine forest. *Can. J. Bot.* **63**, 1436—1443.
- ROBERTS, T. M., T. A. CLARKE, P. INESON & T. R. G. GRAY, 1980. Effects of sulphur deposition on litter decomposition and nutrient leaching in coniferous forest soils. In: HUTCHINSON, T. C., & M. HAYAS (eds.), *Effects of Acid Precipitation on Terrestrial Ecosystems*. Plenum Press, New York, 381—393.
- RUSCHMEYER, O. R., & E. L. SCHMIDT, 1958. Cellulose decomposition in soil burial beds. II. Cellulolytic activity as influenced by alteration of soil properties. *Appl. Microbiol.* **6**, 115—120.
- SCHMIDT, E. L., & O. R. RUSCHMEYER, 1958. Cellulose decomposition in soil burial beds. I. Soil properties in relation to cellulose degradation. *Appl. Microbiol.* **6**, 108—114.
- STRAYER, R. F., & M. ALEXANDER, 1981. Effects of simulated acid rain on glucose mineralization and some physiochemical properties of forest soils. *J. Environ. Qual.* **10**, 460—465.
- C. J. LIN, & M. ALEXANDER, 1981. Effect of simulated acid rain on nitrification and nitrogen mineralization in forest soils. *J. Environ. Qual.* **10**, 547—551.
- TAMM, C. O., G. WIKLANDER & B. POPOVIC, 1977. Effects of application of sulphuric acid to poor pine forests. *Water Air Soil Pollut.* **8**, 75—87.
- TATSUYAMA, K., H. YAMAMOTO, T. SHIOTA & H. EGAWA, 1981. Measuring cellulose decomposition using Benchkote-paper, for the estimation of soil pollution with copper. *Experientia* **37**, 131.
- WAINWRIGHT, M., 1980. Effect of exposure to atmospheric pollution on microbial activity in soil. *Plant and Soil* **55**, 199—204.
- WHITE, J. W., F. J. HOLBEN & C. D. JEFFRIES, 1949. Cellulose decomposing power in relation to reaction of soils. *Soil Sci.* **68**, 229—235.
- ZAR, J. H., 1974. *Biostatistical Analysis*. Prentice-Hall, Englewood Cliffs, N.J.

Addresses of the authors: Dr. R. J. F. BEWLEY, BioTechnica Ltd., 5 Chiltern Close, Cardiff, Wales CF4 5DL, U. K. and Prof. Dr. D. PARKINSON, Kananaskis Centre for Environmental Research, The University of Calgary, 2500 University Drive N.W., Calgary, Alberta, Canada T2N 1N4.

**Synopsis:** *Original scientific paper*

BEWLEY, R. J. F., & D. PARKINSON, 1985. Sensitivity of certain soil processes to acid deposition. *Pedobiologia* **29**, 73—84.

The mineralization of cellulose, urea and casein was monitored in boreal forest soils subjected to varying degrees of acid deposition from a "sour gas" plant. Over approximately one year, 83 %, 91 % and 93 % of the cellulose present on plastic-backed paper strips was decomposed in soils 2.8 km (site 1), 6.0 km (site 2), and 9.6 km (site 3), respectively from the sour gas plant. There was a significant reduction in the rate of CO<sub>2</sub> evolution from F/H material from site 1, unamended, or amended with cellulose or urea compared with similarly-treated samples from sites 2 or 3. The differences between sites 2 and 3 were not significant. A slower rate of CO<sub>2</sub> evolution from cellulose-amended mineral soils of both sites 1 and 2 was observed, compared with site 3. There was also a reduction in the rate of urea decomposition in the mineral soil with increasing acid deposition although this was significant only at site 1, and only during the early stages of decomposition. The retardation in casein decomposition at sites 1 and 2 was more pronounced, however, and resulted from both a longer period before mineralization occurred, and once underway, a slower rate of CO<sub>2</sub> evolution compared with site 3. A similar trend was observed in the ammonification of added casein, although there was little difference between sites in the small amount of ammonium formed in unamended soil after 24 days of incubation. No significant nitrification took place in either casein-amended or unamended mineral soils.

**Key words:** Acid deposition, cellulose, casein, urea, ammonification, mineralization, boreal forest soils.